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Adverse reactions to co-trimoxazole in HIV infection: a reappraisal of the glutathione-hydroxylamine hypothesis

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It is postulated that the unstable hydroxylamine metabolite of sulphamethoxazole is responsible for the adverse reactions to co-trimoxazole and in HIV infection systemic glutathione deficiency leads to a reduced capacity to counteract the hydroxylamine toxicity. This hypothesis has been investigated by studying the metabolism of sulphamethoxazole and assessing glutathione status in HIV infection in order to explore the modification of treatment. It is concluded that the toxicity of plasma sulphamethoxazole hydroxylamine is counteracted by normal glutathione concentrations as is the case in HIV-seropositive patients, but that increased oxidation within certain cells in HIV infected individuals may possibly give rise to increased concentrations of reactive intermediates of sulphamethoxazole.

Sulphametrole and sulphamethoxazole have similar half-lives but are metabolized differently: *in vivo* no oxidised metabolites of sulphametrole could be detected. In a retrospective study the rate of adverse reactions to trimethoprim-sulphametrole appeared to be in the lower range of those reported for trimethoprim-sulphamethoxazole indicating that the combination of trimethoprim-sulphametrole may be more favourable. The ratio of trimethoprim:sulphonamide is 1:5, but *in-vitro* studies with *Toxoplasma gondii* indicate that because of the synergic effect of both agents the dose of sulphonamide is possibly unnecessarily high.

Introduction

The combination of trimethoprim and sulphamethoxazole (co-trimoxazole) is an important drug for the treatment and primary and secondary prophylaxis of *Pneumocystis carinii* pneumonia (PCP) in patients with infection caused by the human immunodeficiency virus (HIV). However, the administration of co-trimoxazole in these patients is often hampered because the frequency of adverse reactions to this drug in HIV infected patients is much higher compared with seronegative individuals (Kovacs *et al.*, 1984). These side-effects, which are often labeled as 'allergic', consist of rash, fever and damage to liver parenchymal cells. Although HIV seropositive individuals are more susceptible to side-effects similar to those associated with a number of other, unrelated drugs (reviewed by Coopman *et al.*, 1993; Koopmans *et al.*, 1995), this review focuses on the side-effects of co-trimoxazole.

Some years ago, we hypothesized that this increased frequency of toxicity to co-trimoxazole is caused by a combination of two factors (van der Ven *et al.*, 1991, 1994b). First of all, the hydroxylamine derivative of sulphamethoxazole is an unstable metabolite that can undergo auto-oxidation to become a protein-reactive nitroso derivative, and this substance could be responsible for the adverse reactions to co-trimoxazole (Shear *et al.*, 1986; Rieder *et al.*, 1988, 1989). This means that the sulphonamide component of co-trimoxazole is largely responsible for the side-effects, although the oxidation of trimethoprim could possibly also lead to hydroxylamine formation. Secondly, HIV infected individuals have a systemic glutathione deficiency (Buhl *et al.*, 1989; Eck *et al.*, 1989). This deficiency implies that there is a reduced capacity to prevent auto-oxidation of the unstable sulphonamide intermediates, and to scavenge reactive compounds. As a consequence, there is an increased exposure of cells to toxic intermediates and this could lead to the adverse reactions.

The hypothesis was the subject of a series of investigations carried out by our group. The investigations comprised studies of the metabolism of sulphamethoxazole and other sulphonamides, the assessment of the glutathione status in HIV-infected patients and the exploration of treatment modifications.

Sulphamethoxazole metabolism

Sulphamethoxazole is extensively metabolized *in vivo*; its metabolic pathways involve acetylation, oxidation and glucuronidation (Vree & Mekster, 1987; Vree *et al.*, 1994). Oxidation takes place at different positions of the molecule: the unstable, reactive hydroxylamine metabolite is formed by oxidation of the N4 nitrogen atom while oxidation of the C5 methyl group leads to the stable 5-hydroxy metabolite. Formation of both metabolites by cytochrome P450-mediated reaction was demonstrated *in vitro* using human liver microsomes. *In vitro* more sulphamethoxazole was N-hydroxylated than 5-hydroxylated (van der Ven *et al.*, 1995a).

The pharmacokinetics of sulphamethoxazole and its metabolites were reported in healthy volunteers after a single dose (Vree *et al.*, 1994, 1995). The patterns of the formation and elimination of sulphamethoxazole hydroxylamine were similar to the other metabolic products, and the renal excretion of sulphamethoxazole hydroxylamine accounted for 2.4% of the recovered dose (van der Ven *et al.*, 1994c). The mean residence time of the hydroxylamine metabolite was 5.5 h; the renal clearance amounted 4.39 L/h. Thus, under physiological conditions most sulphamethoxazole hydroxylamine seems to be stable, escapes the liver and is excreted by the kidney.

Next, the pharmacokinetics of sulphamethoxazole and its metabolites were also investigated in HIV-seropositive individuals after a single oral dose (van der Ven *et al.*, 1995b). Sulphamethoxazole hydroxylamine is the only compound for which the urinary recovery was found to be different from healthy volunteers. The impaired recovery of sulphamethoxazole hydroxylamine in HIV-seropositive patients (50% reduction) may be explained by secondary metabolism to the protein reactive nitroso derivatives. At the time of the investigations, it was not clear whether this auto-oxidation occurred in the urine *ex vivo* or *in vivo* because of glutathione deficiency. Because the urinary excretion of all other metabolites of sulphamethoxazole, including 5-hydroxy sulphamethoxazole and N4-acetyl-5-hydroxy-sulphamethoxazole, was similar between HIV-seropositive

individuals and healthy controls, we concluded that there was no difference in oxidation, acetylation and glucuronidation (van der Ven *et al.*, 1994b). Taken together, we conclude that sulphamethoxazole hydroxylamine is indeed being formed during treatment with sulphamethoxazole by both normal individuals and HIV-seropositive patients.

Although the cytotoxic potential of hydroxylamines of sulphonamides can be demonstrated *in vitro* (Rieder *et al.*, 1988, 1989), the relative toxicity of these compounds, as compared with the parent compounds (which also exert cytotoxicity; A. J. A. M. van der Ven, unpublished observations), has not been assessed in terms of the relative concentrations *in vivo*.

Glutathione status

Glutathione is the main intracellular defense against oxidative stress and is of major importance for xenobiotic metabolism. Intracellular concentrations of glutathione are high, while plasma concentrations are low. Several groups have reported decreased glutathione concentrations in HIV infection, in particular in plasma, broncho-alveolar lavage, peripheral blood mononuclear cells (Buhl *et al.*, 1989; Eck *et al.*, 1989), and also in CD4+ and CD8+ T lymphocytes (Roederer *et al.*, 1991). Based on these investigations, it was concluded that HIV infection is associated with a systemic glutathione deficiency. Glutathione concentrations in CD4+ and CD8+ T lymphocytes were determined using monochlorobimane as a fluorescent label. However, when we evaluated this method, we found that many variables influenced the cellular fluorescence, including the presence of alternative metabolic pathways for monochlorobimane and the rapid excretion of glutathione-bimane conjugate out of the cell. We concluded that this label cannot be used to assess glutathione levels in these cells (van der Ven *et al.*, 1994a).

High performance liquid chromatography is a validated method to measure glutathione concentrations and was employed to measure free and protein-bound glutathione concentrations in HIV-seropositive individuals and healthy controls. Both forms of glutathione were determined because free glutathione is important for the maintenance of thiol redox status, while increased concentrations of protein-bound glutathione indicate oxidative stress. We found that free intracellular glutathione concentrations in erythrocytes, CD14 cells and CD4 cells were not different between HIV-seropositive individuals and healthy controls (A. J. A. M. van der Ven, H. Blom, W. H. M. Peters, L. E. H. Jacobs, I. J. O. Verver, P. P. Koopmans, P. Demacker & J. W. M. van der Meer, unpublished observations). In addition, we found that plasma concentrations of free glutathione were also not different. Based on these findings, we concluded that HIV infection is not associated with a systemic glutathione deficiency. It was found, however, that the protein-bound form of glutathione was increased, particularly in CD4 cells of HIV seropositive subjects which strongly suggests the presence of increased oxidative stress.

So far, we have concluded that the toxicity of sulphamethoxazole hydroxylamine in plasma is counteracted by normal glutathione concentrations, not only in normal controls but also in HIV-seropositive patients. It could be speculated that in these patients, the presence of increased oxidation within certain cells may lead to increased concentrations of reactive intermediates of sulphamethoxazole. It is clear that further research is needed in this area.

Treatment modification

Treatment modification with regard to the choice of the sulphonamides was also investigated. We explored the metabolism of a number of different sulphonamides to try and select a sulphonamide that would generate less hydroxylamine. Ten different sulphonamides were incubated *in vitro* with human liver microsomes. These experiments indicated that all sulphonamides tested generated stable as well as unstable oxidised metabolites, but not all to the same extent. The generation of oxidised products was least for sulphametrole. *In vivo* the rate of oxidation between the various sulphonamides also differs; the same holds true for the rate of acetylation and glucuronidation (Vree & Hekster, 1987). It is generally accepted that the acetylated compound is non-toxic (Rieder *et al.*, 1991); thus, a sulphonamide that is accessible for acetylation but not for oxidation may be more favourable than an extensively metabolized compound like sulphamethoxazole. The acetylation of sulphametrole *in vivo* has been previously reported (Hekster *et al.*, 1981), and to date is the only metabolic reaction. Re-examination of sulphametrole metabolism using mass spectrometry did not indicate the presence of any oxidised metabolite in the urine of healthy controls and HIV seropositives (W. Welz, personal communication). Since a stable hydroxy metabolite could not be detected, it is likely that sulphametrole is not accessible for cytochrome P450 mediated reactions *in vivo*. Like sulphamethoxazole, sulphametrole is commercially available in a 5:1 dose ratio with trimethoprim (Co-soltrim). Sulphamethoxazole and sulphametrole have similar half-lives (10 h).

From the inability to detect hydroxylamine metabolites of sulphametrole, we were eager to know whether administration of sulphametrole would meet with fewer adverse effects, especially in HIV seropositive patients. This was supported by a report in the literature, in which sulphametrole did not appear to be associated with haemolysis when administered in a population with a high prevalence of glucose-6-phosphate dehydrogenase deficiency (Plummer *et al.*, 1983). These patients lack the capacity to keep glutathione in its reduced form, especially in the red blood cells; sulphamethoxazole is known to cause haemolysis in these patients (Beutler, 1991). Recently, we performed a retrospective analysis of 58 HIV infected patients treated for PCP with Co-soltrim in two centres. The analysis showed that only skin reactions or fever were indications to alter medication and that adverse reactions occurred less frequently than has been reported previously for co-trimoxazole (A. J. A. M. van der Ven, A. Rieger, A. Brahten, R. Reiman, T. B. Vree, P. P. Koopmans, J. W. M. van der Meer, personal observations). These results would justify a prospective controlled study comparing the frequency of side-effects of sulphamethoxazole and sulphametrole in HIV-infected patients.

Since our hypothesis points to a concentration-dependent mechanism rather than to an allergic (i.e., concentration-independent) mechanism, dose reduction would be an option. Such a strategy finds some support from the study of Schneider *et al.* (1992), which demonstrated that a relatively low dosage of co-trimoxazole (480 mg daily) for PCP prophylaxis in HIV-positive patients was associated with delayed occurrence of side-effects than a higher dosage (960 mg daily). In view of this dosage dependency, we investigated the optimal dosage ratio of sulphonamide and dihydrofolate reductase (DHFR) inhibitors (trimethoprim and pyrimethamine). At present the achieved plasma concentration of the sulphamethoxazole (100–200 mg/L) is much higher than that of the DHFR inhibitor (for trimethoprim 5–10 g/L). The dosage recommendations are

based on inhibitory concentrations of each compound separately without taking into account the synergic effect of the combination. This synergic effect was demonstrated in an in-vitro study using *Toxoplasma gondii*. We found that the 50% inhibitory concentration of sulphamethoxazole reduced from 500 to 0.05 g/L when pyrimethamine 0.05 g/L was added to the medium; the plasma concentrations of pyrimethamine obtained with a once weekly dose of 75 mg are around 0.2 g/L (A. J. M. van der Ven, E. M. E. Schoondermark van de Ven, W. Camps, W. J. G. Melchers, P. P. Koopmans, J. W. M. van der Meer & J. M. D. Galama, personal observations). These findings could imply that the present dosage recommendations for sulphonamides, when combined with trimethoprim or pyrimethamine, are too high, and thereby result in unnecessary increased toxicity. Reduction of the toxicity of the current first line treatments for *P. carinii* and *T. gondii* by selection of a less toxic sulphonamide used with a DHFR inhibitor at an adjusted dosage ratio should be investigated.

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